

## Restriction Digest Protocol

1. Spin down all enzymes and DNA in the centrifuge by pressing the short button until max speed is reached.
2. Put all enzymes, buffers, and DNA on ice.
3. Add 1  $\mu\text{g}$  of DNA to the appropriately labeled tube. Add distilled water to 43  $\mu\text{L}$ .
4. Pipet 5  $\mu\text{L}$  of NEB Buffer 2 to each tube.
5. Add 1  $\mu\text{L}$  of each of the desired enzymes.
6. Place the samples in the 37°C water bath for 1-2 hours.
7. Perform gel purification using the QIAGEN gel purification kit and corresponding protocol.

Component	Amount
DNA	500 ng
dH <sub>2</sub> O	to 32 $\mu\text{L}$
NEB Buffer 2	5 $\mu\text{L}$
Enzyme 1	1 $\mu\text{L}$ EcoRI
Enzyme 2	1 $\mu\text{L}$ SpeI